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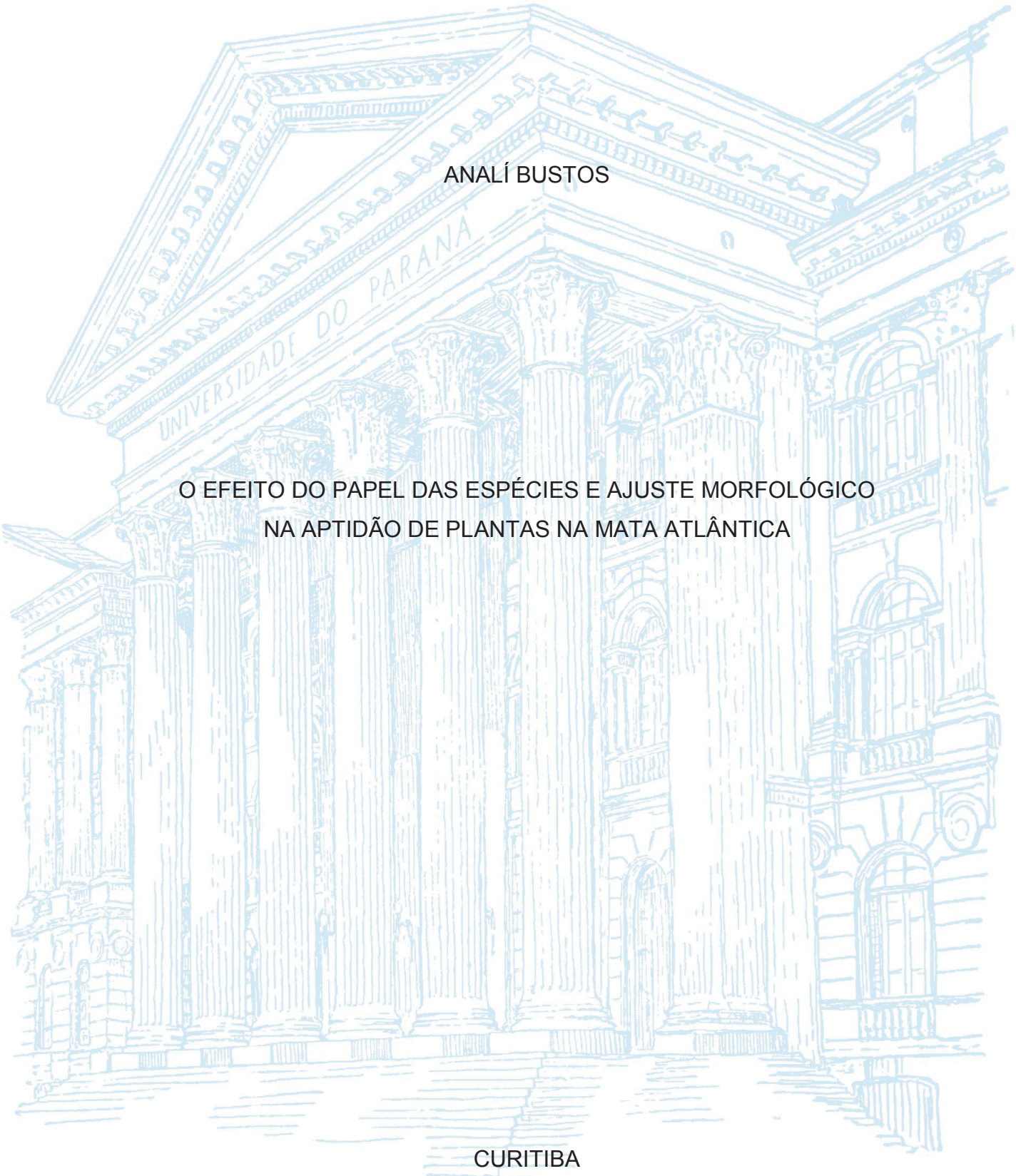

O EFEITO DO PAPEL DAS ESPÉCIES E AJUSTE MORFOLÓGICO NA APTIDÃO DE PLANTAS NA MATA ATLÂNTICA

CURITIBA

2020

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O EFEITO DO PAPEL DAS ESPÉCIES E AJUSTE MORFOLÓGICO NA APTIDÃO DE PLANTAS NA MATA ATLÂNTICA

Dissertação apresentada ao curso de Pós-Graduação em Ecologia e Conservação, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Ecologia e Conservação.

Orientadora: Profa. Dra. Isabela G. Varassin

Co-orientadora: Profa. Dra. Catherine Graham

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TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ECOLOGIA E CONSERVAÇÃO da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **ANALI BUSTOS**, intitulada: **O EFEITO DO PAPEL DAS ESPÉCIES E AJUSTE MORFOLÓGICO NA APTIDÃO DE PLANTAS DA MATA ATLANTICA**, sob orientação da Profa. Dra. ISABELA GALARDA VARASSIN, após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de Mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

Curitiba, 17 de Fevereiro de 2020.

ISABELA GALARDA VARASSIN
Presidente da Banca Examinadora

CAMILA SILVEIRA DE SOUZA
Avaliador Externo (UNIVERSIDADE FEDERAL DO PARANÁ)

LEANDRO FREITAS
Avaliador Externo (UNIVERSIDADE FEDERAL DO PARANÁ)

A meus pais **Aldo** e **Amanda** e minha irmã **Anabel**, por me ajudar cumprir meus sonhos como se fossem os seus.

À memória de meu querido amigo **Iván**.

A eles dedico esta dissertação

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O correr da vida embrulha tudo.
A vida é assim: esquenta e esfria,
aperta e daí afrouxa,
sossega e depois desinquieta.
O que ela quer da gente é *coragem*

Guimarães Rosa

RESUMO

A aptidão das plantas é afetada pela limitação polínica e ocorre quando as plantas produzem menos frutos e/ou sementes do que produziriam recebendo uma adequada quantidade e qualidade de pólen. Existem diversos fatores que podem afetar a eficiência da polinização, porém são escassos os estudos que vinculam a estrutura da rede com a funcionalidade ou aptidão das espécies vegetais. Aqui testamos, tanto no nível de espécies quanto de flores individuais, se a centralidade, a seletividade de interações e o ajuste morfológico planta-polinizador estão relacionadas à aptidão de 14 espécies de plantas na Mata Atlântica. Na rede baseada em espécies, não encontramos relação entre centralidade, seletividade de interações e ajuste morfológico e a aptidão. Porém, em uma análise mais profunda na rede baseada em flores, encontramos um efeito positivo entre o aumento da centralidade das flores e seu número de sementes. O ajuste morfológico não foi significativamente relacionado à aptidão, portanto, o nível de correspondência entre os atributos de bicos e corolas não afetou a produção de sementes. Nosso trabalho mostra as diferenças nos resultados reprodutivos de flores individuais em redes de polinização e sugerem que a posição dentro da rede pode ter importantes implicações na eficiência da polinização.

Palavras-chave: Aptidão, Mata Atlântica, polinização, redes de interação.

ABSTRACT

Plant fitness is affected by pollen limitation, which occurs when plants produce fewer fruits and/or seeds than they would if they received adequate amounts and quality of pollen. There are diverse factors that can affect the efficiency of pollination, but there are still few studies linking network structure and functionality or plant fitness. Here we tested, at both the level of species and individual flowers, if centrality, interaction selectivity and plant-pollinator trait-matching were related to plant fitness for 14 plant species in the Atlantic Forest. We did not find a relationship between centrality, interaction selectivity and trait-matching and plant fitness when investigating networks at the species level. However, for network-characteristics at the flower-level we found a significant positive relationship between flower centrality and seed production. Trait-matching was not significantly related to fitness, thus the level of matching between bills and corolla's morphology didn't affect seeds production. Our findings show differences in the reproductive outcomes of individual flowers in pollination networks and suggest that the position within the network may have important implications to pollination effectiveness.

Keywords: Atlantic Forest, interaction networks, plant fitness, pollination.

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SUMMARY

1. INTRODUCTION

Plant fitness, i.e. the quantity and quality of seeds produced, is affected by pollen limitation. This limitation occurs when plants produce fewer fruits and/or seeds than they would if the received additional pollen (Ashman *et al*., 2004; Harder & Aizen, 2010). In animal-pollinated plants, the abundance, efficiency and behaviour of flower visitors determine the efficiency of pollination and consequently, plant fitness (Knight *et al*., 2005; Castro *et al*., 2015). Besides that, both ecological and phenotypic pollination specialization, i.e. the number of species from different functional groups that visit a given plant and flower morphological traits, are the factor that best explained the occurrence of pollen limitation (Wolowski *et al*., 2014). Although some studies have been done considering reproductive outcomes of interactions (Gómez & Perfectti, 2012), studies linking network structure and functionality or fitness are still scarce, probably due to the difficulty in obtaining replicated field data on fruit or seed production for a great number of species (Tur *et al*., 2013; Lázaro *et al*., 2019). Our study was designed to fill this gap. Using the number of seeds produced as fitness measurement, we evaluated whether the position of a plant species and flowers within a plant-pollinator network, its interaction selectivity, and plant-pollinator trait-matching, affect plant fitness in the Atlantic Forest.

Plant fitness can be measured in different ways. In a review about pollen limitation and plant reproduction, Knight *et al*. (2005) found that fruit set was the most common response variable used. However, the number of seeds per plant is the most appropriate response variable for most questions related to the study of pollen limitation, as it measures the effect on maternal fitness of an individual plant (Dudash & Fenster, 1997; Ashman *et al*., 2004; Knight *et al*., 2005).

For pollination networks, the position of a species in the network can be measured by centrality indices, which describes how connected a plant species is to the rest of the co-occurring species due the number of interactions it establishes or due to shared floral visitors (Nooy *et al*., 2005; Dormann, 2011). The most abundant species, as well as the most generalist ones, usually occupy central positions in ecological networks; thus, central species are proportionally more important for the stability and functioning of the whole system than peripheral species, being crucial to predict responses to disturbances (Martin González *et al*., 2010; Sazima *et al*., 2010; Gómez & Perfectti, 2012). Lázaro *et al*., (2019) highlighted that one way to understand the validity of species-level network metrics when predicting functionality, could be assess their relationship with fitness. Initial studies showed that individuals in central positions have higher fitness than those in peripheral positions (Gómez & Perfectti, 2012; Tur *et al*., 2013) but whether this also occurs in the interspecific context of common community-level networks is yet unknown.

If plant-pollinator evolution is the result of a co-adaptive process, interactions between plants and pollinators would be expected to become more specialized (Aigner, 2001). However, several studies show that many plants are visited and pollinated by a wide and diverse range of animal species that overlap in its floral use (Waser *et al*., 1996; Gómez *et al*., 2007). Interaction selectivity in ecological networks measures the deviation of niche overlap observed between species at the community-level from that expected as a function of the frequency of interaction between species (Blüthgen *et al*., 2006). Lower plant selectivity might be a good strategy in scenarios where pollinator abundances and interactions fluctuate across time (Kremen *et al*., 2002, Klein *et al*., 2003; Hoehn *et al*., 2008). Actually, a positive effect of pollinator diversity on pollination services has been detected (Hoehn *et al*., 2008) indicating that greater generalization - in terms of species diversity - tends to result in greater fitness (Tur *et al*., 2013). If this generalization effect also influences interaction selectivity –

generalization in terms of interactions – then we expect that lower interaction selectivity will result in higher fitness. On the other hand, several previous theoretical (Pauw, 2013) and empirical studies (Poisot *et al*., 2013; Valdovinos *et al*., 2016; Magrach *et al*., 2019) have shown that lower pollinator niche overlap results in greater plant fitness. These findings suggest that a certain degree of interaction selectivity might be necessary to determine differences in reproductive outcomes, or more specifically, to ensure a proper conspecific pollen deposition (Flanagan *et al*., 2009; Magrach *et al*., 2019).

A likely cause of selectivity in plant-pollinator interactions is trait-matching, a pattern expected either through reciprocal co-evolutionary processes or through ecological fitting between pairs of species with independent trait evolution (Guimarães *et al*., 2011; Maglianesi *et al*., 2014). The reciprocal morphological adaptations, such as between plant corollas and pollinator mouthparts, can result in species with similar traits interacting more frequently (Weinstein & Graham, 2017). Besides that, species tend to interact with species whose traits allow them to exploit resources more efficiently (Maglianesi *et al*., 2014) and an interaction could not happen if there is a morphological mismatch among potential interacting partners (Jordano *et al*., 2003; Dehling *et al*., 2014). In most hummingbird–flower communities, there is a subset of flowers with long, curved corollas visited by only one or a few long- and curve-billed birds (Maglianesi *et al*., 2014; Zanata *et al*., 2017). This indicates that the increased range of bill and corolla lengths in hummingbird–flower networks may contribute to reduced niche overlap and increased community-level specialization (Cotton, 1998; Maruyama *et al*., 2014; Maglianesi *et al*., 2015; Zanata *et al*., 2017). The consequences of a strong trait-matching between flowers and pollinators can result in increased fitness of both, providing quality pollination services as well as a high efficiency in resource intakes (Dohzono *et al*., 2011; Maglianesi *et al*., 2014).

Birds play an important role in plant reproduction in tropical biomes, acting as dispersers or pollinators for many species (Snow, 1981). An important group of pollinators unique in America are hummingbirds; are one of the largest bird families (Trochilidae) with about 360 species (Schuchmann, 1999). In Brazil there are 84 species, of which 16 are endemic and 43 occur in the Atlantic Forest (Piacentini & Ribenboim, 2017). Hummingbirds are an important group of pollinators of Neotropical forests (Bawa, 1990), pollinating from 10 to 15 percent of plant species at the Atlantic Forest (Buzato *et al.*, 2000). Besides that, among nectarivourous birds hummingbirds are the most phenotypically specialized (Zanata *et al.*, 2017).

Our objective is to evaluate whether the position of plants species and individual flowers into the network and plant-pollinator trait-matching affect the fitness of plant species in the Atlantic Forest. Specifically, we evaluated whether plant fitness is related to plant and flower (i) centrality; (ii) interaction selectivity; and (iii) plant-hummingbird trait-matching. Thus, we propose a set of predictive hypotheses: Plant species and individual flowers within each plant species will present higher fitness when they (i) are more central within the network, and (ii) have lower interaction selectivity. Plant species will have higher fitness when there is (iii) greater trait-matching between hummingbird bill and plant corolla length.

2. METHODS

Study Site

We carried out the fieldwork in two protected areas, Estação Biológica de Santa Lúcia (19º 57'S, 40º32' W) and Reserva Biológica Augusto Ruschi (19°54'20"S, 40°33'44"W), Santa Teresa municipality, southeastern Brazil (Figure 1). The elevation ranges from 600 to 900 mamsl, with an average annual maximum temperature of 26,3°C and an average annual minimum of 14,3°C (Thomaz & Monteiro, 1997). The area is covered by tropical rain forest (Mendes & Padovan, 2000).

Plant-pollinator interactions

We sampled three 1,5 km by 10m transects for five months, from March to July 2019. Each month we sampled plant-pollinator interactions using time-lapse cameras (Plotwatcher Pro) placing 12 cameras in front of flowering plants, for three days. Our survey included a total of 14 different plant species potentially visited by hummingbirds that were selected based on their availability, i.e. most abundant plant species. Those species represent 41% of 34 species that are visited by hummingbirds in the sampled area (unpublished data).

Time-lapse cameras recorded pictures every second from dawn to dusk (~12 hours) and then videos were processed by the Deep Meerkat program (Weinstein, 2015). This software was able to detect frames with hummingbirds and butterflies. From the pictures, we managed to identify the pollinators at each flower and the number of visits by each species. We only counted the legitimate interactions, that is, those where the pollinators visited the flowers through the corolla opening. To count the interactions at a given flower, we considered the interactions detected by a given pollinator as independent when a new interaction occurred after 20 seconds interval.

Plant fitness

To explore the relationship between visits and seed set for each flowering plant, we marked up to five flowers per plant to follow at a given flower 1- the identity of functional group of pollinators: hummingbirds and/or butterflies (*sensu* Fenster *et al*., 2004), 2- the identity of pollinators (species), 3- the number of visits made by each pollinator species and 4- seed set. We surveyed each flower until the fruits were ripe, and then we counted the number of seeds per fruit. We assessed the fitness outcomes in two ways: the first one was at species level, as an average species measure of fitness calculated as the mean between the number of seeds produced by each individual, divided by the maximum number of seeds produced by one individual of that species, as follows:

$$\text{Fitness Sp1} = \overline{X}\left(\frac{\text{N° seeds individual 1}}{\text{N° seeds}_{max}\text{ in the Sp 1}}\right)$$

This standard measure allowed all the focal plant species to be compared. The second one was measured at flower level, as direct counts of the number of seeds per fruit.

Centrality and interaction selectivity

Based on the records of interactions we built two adjacency matrices weighted by interaction frequency (number of visits made by pollinators). To evaluate species network roles, a species-based matrix was built with plants in rows and pollinators in columns; we then calculated two measurements of centrality (*DC, CC*$_w$) and interaction selectivity (*d*') for each plant species, as explained below. To evaluate the role of a given flower in a network, we built a flower-based matrix where flowers of each species were placed in the rows and pollinators in the columns. We calculated two measurements of centrality (*DC, CC*$_w$) and

interaction selectivity (*d*') for each flower within each plant species. Network metrics were calculated using the package "bipartite" (Dormann *et al*., 2008) in R (R Core Team, 2019).

Degree centrality (*DC*) is defined by the number of links that are connected with a node (Jordán *et al*., 2006). A plant species with high degree centrality interacts with many pollinators in a network, thus a species is central when it is well connected (Martín González *et al.,* 2010). It provides a description of network connectivity based on the individual components. To *i* species DC_i is calculated as:

$$DC_i = k_i / (\text{N-1})$$

where N is species richness and *k* is *i*'s number of interactions (Jordán *et al*., 2006).

Closeness centrality (*CC*) is a measure quantifying the shortest number of direct and indirect interactions between one species and all the other species in the network (Jordán *et al*., 2006). A plant species with high closeness centrality shares many pollinators with many other plants in a network (Mello *et al*., 2015). To *i* species this metric is defined:

$$\mathbf{CC_i} = \frac{\mathbf{N} - 1}{\sum_{\mathbf{j=1}}^{\mathbf{N}} \mathbf{d_{ij}}}$$

where N is species richness and *dij* is the length of the shortest path between species *i* and *j* (Jordán *et al.,* 2006). In our analysis we used weighted closeness centrality (CC_w), that is, the links are weighted by the frequency of these interactions in the network (Dorman, 2011; Opsahl *et al*., 2010). Because CC_w is a weighted metric, we tested if closeness centrality is related to interaction frequency at species level.

Interaction selectivity (*d'*) describes the deviation of niche overlap observed between species at community level from that expected as a function of the frequency of interaction between species (Blüthgen *et al*., 2006). Therefore, it detects the exclusivity of interactions

that is not predicted by the chance of encounter between pairs of species. The values of this metric range between 0 and 1, lowest and extreme selectivity, respectively (Blüthgen *et al*., 2010).

Plant-hummingbird trait-matching

To evaluate the effect of trait-matching on plant fitness, we used both plant and hummingbird traits. For each plant species we collected five flowers of five different individuals and we measured effective corolla length, from the base to the opening of floral tube (*sensu* Wolf *et al*., 1976). For hummingbirds we used bill length data (Zanata *et al*., 2019), measured as the chord of exposed culmen, i.e. from the tip to the anterior extension of the feathers on the bill. Trait-matching was calculated as the difference between corolla and bill length for each interacting pair of species, and then for a given plant species, we calculated the mean trait-matching considering all the pollinator species it interacted. We named this variable as Delta trait-matching, that is the degree of mismatch between each plant species with all its pollinators divided by the total number of pollinator species that visited this plant species. It was calculated as follows:

$$\Delta\text{trait-matching} = \frac{\Delta(\text{p1h1}) + \Delta(\text{p1h2}) + \Delta(\text{p1h3})}{\text{N° of pollinator species}}$$

We deposited plant vouchers for each species at the MBML herbarium (Museu de Biologia prof. Mello Leitão) at Instituto Nacional da Mata Atlântica. We evaluated trait-matching only for the species-based network because we did not have the individual measurements for each interacting flower visited.

Statistical analysis

First, to determine if the functional groups of pollinators should be included as another fixed factor in the subsequent analyses, we evaluated the influence of the functional group on plant fitness. Previous experimental studies have shown a positive relationship between the diversity of functional groups of pollinators and seed set (Hoehn *et al*., 2008); interspecific differences in these functional groups allow a high niche complementarity resulting in enhanced fruit set (Hoehn *et al*., 2008). Thus, in our study we expected that the interactions of hummingbirds plus butterflies produced greater seed set than either group separately. Using a *t*-test (R Core Team, 2019) we compared the production of seeds between flowers that were visited only by hummingbirds and flowers visited by hummingbirds and butterflies.

We assessed if plant fitness is explained by centrality, interaction selectivity and trait-matching in the species-based network, performing simple univariate analyses. When normality assumption was not fulfilled, the logarithm or square root transformations were applied to the variables. To determine if closeness centrality and interaction selectivity were related to plant fitness, we used linear regression analysis, with the "lm" function (R Core Team, 2019). For degree centrality the normality assumption was not met even after transformations. Therefore, we then used a linear model with permutation analyses to relate degree centrality and plant fitness, with the "lmp" function (R Core Team, 2019).

In more detailed analysis within species, in the flower-based network, we tested if the number of seeds produced by a given flower is explained by its centrality and interaction selectivity. First we analyzed each metric individually, then we evaluated two models with different combinations of fixed parameters, using plant species (sp) as a random factor, as follows:

Model 1: Number of seeds ~ (1| sp) + d' + DC

Model 2: Number of seeds ~ (1| sp) + d' + CC_w

Where: sp = plant species; *d'* = interaction selectivity; *DC* = degree centrality; CC_w = weighted closeness centrality.

We used generalized linear models (GLM) with Poisson distribution, using the function "glm" (R Core Team, 2019). Model selection was made with the corrected Akaike Information Criterion (AICc) choosing the most explanatory model, which is the model with the lowest AICc value (Burnham & Anderson, 2004).

3. RESULTS

After 2016 hours of recording, 835 plant-pollinator interactions were observed among 14 plant species distributed in four plant families (72% Bromeliaceae, 14% Campanulaceae, 7% Acanthaceae, 7% Malvaceae) and 8 pollinator species belonging to three families (50% Trochiliidae, 37% Heliconinae, 13% Riodinidae) (Table 1). Most interactions were done by hummingbirds (93%) and only 7% by butterflies.

Only eight plant species were visited simultaneously by hummingbirds and butterflies (Figure 2); no plant species was visited only by butterflies. In these species we observed 578 plant-pollinator interactions, 520 belonged to hummingbirds and 58 belonged to butterflies. Plant species did not show a significant difference on fitness when they were visited by hummingbirds compared to when they were visited by hummingbirds plus butterflies (t = 0.27, p = 0.7854, df = 13) (Figure 3). Given these results we did not consider the variable "functional group" and only considered hummingbird interactions on the next analyses. Thus, the resulting interaction network had 14 plant species and four hummingbird species (Figure 4). For this network, plant fitness was positively correlated with the frequency of hummingbird visits (adjusted r^2= 0.41, p= 0.008, df = 12) (Figure 5).

Species-based network analyzes

In the species-based network plant fitness was neither related to degree centrality (adjusted r^2= 0.08, p= 0.9425, df = 12) or closeness centrality (adjusted r^2= 0.05, p= 0.5895, df = 12). Interaction frequency explained 33% of closeness centrality variation (p= 0.01, df= 12, r^2=0.33; Figure S1). No significant relationship between interaction selectivity and plant fitness was detected (adjusted r^2= -0.07, p= 0.8143, df = 12).

Flower-based network analyzes

Our results showed that model 2, including interaction selectivity and closeness centrality, was the best fit to our data (lowest AIC_C value = 7879.4) (Table 3). Interaction selectivity (*d'*) and closeness centrality (CC_w) together explained 6% (marginal $r^2 = 0.06$) of the variability observed in flower fitness. Whereas interaction selectivity didn't affect the number of seeds produced ($p = 0.613$) (Figure 6a, 7), flowers with higher closeness centrality had a greater number of seeds ($p < 0.0001$) (Figure 6b, 7). Contrastingly, model 1, including degree centrality (*DC*) and interaction selectivity (*d'*), had an AIC_C value= 8983.2 (Table 3, A).

Trait-matching

The degree of trait-matching between corolla length and bill length was not related with plant fitness (adjusted $r^2 = -0.0972$, $p = 0.1472$, df = 12) (Figure 8).

4.DISCUSSION

Our results that more central flowers produced more seeds in the flower-based network supports the idea that plant fitness outcomes depend on the position of an individual in interactions networks, and thus links population dynamics to network structure. In the flower-based network the number of seeds per flower increased with closeness centrality. Contrary to our expectations, we did not find any relationship between centrality and interaction selectivity with plant fitness in the species-based network. The relationship between trait-matching and plant fitness was not significant, thus the level of matching between bills and corolla's morphology didn't affect plant reproductive outcomes.

In our study, closeness centrality depicts the importance of the role played by a flower in the larger community due to shared pollinators and its contribution to network cohesiveness (Magrach *et al*., 2019). The effect of centrality on fitness is partially related to the number of interactions done by each flower (i.e. interaction frequency) (Aizen & Harder, 2007) but also due to flower position within the network. This is probably because flowers in central positions, i.e. that share more pollinators, have enhanced conspecific pollen flow resulting in a greater seed set, as found for individuals occupying central network positions (Gómez & Perfectti, 2012; Tur *et al*., 2013) and as for species in central positions (Lázaro *et al*., 2019). Because flower-based networks focus on the centrality within species, central flowers would not be affected by negative effects of closeness centrality on fitness due to heterospecific pollen deposition as reported in some studies (Morales & Traveset, 2008; Muchhala & Thomsom, 2012; but see Tur *et al.,* 2013). Actually, studying individual-based networks for a given species, Gómez & Perfectti (2012) suggest the occurrence of a collective component of fitness, due to the effect of individual flower phenotypes on centrality. This means that fitness responses would not only be due to an individual phenotype, but how this phenotype is related to the phenotype of other flowers or individuals in the network (Gómez & Perfectti, 2012). Plant reproductive outcomes may not always be strongly related to the

observed visitation rates used to build the networks (Dauber *et al*., 2010; Schüepp *et al*., 2014; Lázaro *et al*., 2019); fruits or seeds production could also be related to other factors not considered in this study such as efficiency of pollinators, quality of pollen deposited or competition between pollinator species. These aspects should be addressed more deeply in future research questions.

The unexpected results of absence of effect of interaction selectivity on fitness at both species-level and flower-level reveals flowers with more selective interactions have the same seed set then those flowers with less selective interactions. For those plants, seed set depend more on the number of visits received and number of shared pollinators (CC_w). Indeed, previous studies have found that species in central positions (CC_w) in the network presented less interaction selectivity (Tur *et al*., 2013; Watts *et al*., 2016; Lázaro *et al*., 2019). Besides that, increased interaction selectivity was reported to increase heterospecific pollen transfer (Arceo-Gómez *et al*., 2020). This suggests that next studies could benefit from dedicating a special effort to estimate the degree of correlation between network metrics and not only their separated effects on reproductive success.

We didn't find a significant relationship between plant-hummingbird trait-matching and plant fitness for the species-based network. Stebbins' Most Effective Pollinator Principle (MEPP) formalized the assumption that a plant should always evolve its phenotypic specializations to increase visits by its most effective pollinator (Aigner, 2001) which would result in higher trait-matching. A high degree of trait complementary between bills and corollas shapes has been commonly observed; long-billed and curve-billed hummingbird species prefer plant species with long and curved flowers (Feinsinger & Colwell, 1978; Temeles *et al*., 2009; Maglianesi *et al*., 2014, Weinstein & Graham, 2017). However, though these studies have documented close matches between hummingbird and floral morphologies (e.g., Snow & Snow, 1980; Stein, 1992; Temeles *et al*., 2000), others have reported

hummingbird species visiting plant species with flowers substantially longer or shorter than their bills (Feinsinger, 1976; del Coro Arizmendi & Ornelas, 1990; Cotton, 1998). Our results are consistent with previous studies at the same Atlantic Forest area that showed that the floral and hummingbird bill traits trait were not be correlated (Carneiro Capucho *et al*., 2007). There are some possible explanations for the apparent mismatches between bill morphology and flower morphology in nectar feeding birds. First, only corolla length and sometimes curvature are usually considered and other floral characteristics that may affect foraging abilities are ignored (e.g., Harder, 1985). A hummingbird's ability to use flowers that are longer than its bill may depend on the diameter of the flower's opening: long flowers with narrow openings excluded short-billed birds, whereas long flowers with wide openings can be accessed by short-billed birds (Temeles, 1996). Because we only tested one trait, lengths of the structures, this might have resulted in the weak relationship found. In addition, a low availability of preferred food, combined with competition for this food may result in the incorporation of flowers into the diet that have little correspondence to the forager's feeding morphology (Temeles *et al.*, 2009). This later explanation seems unlikely for the studied area because limited competition was detected among hummingbirds in a related study (Nieto, 2020). These findings in several empirical and theoretical studies, added to our results, indicate that simple observational mismatches between bill and floral phenotypes may not correspond to lower pollination efficiency (Temeles *et al*., 2006; Collins, 2008).

In summary, our findings suggest that individual roles within a mutualistic network can have important dynamic implications, influencing ecosystem functionality as well as its ability to respond to disturbances (Bascompte & Jordano, 2013). Disturbances involving changes at the population level that alter the position of individuals within the networks can result in changes on fitness. Therefore, our results stress that the understanding of plant fitness outcome cannot be accessed without considering the network where each flower is embedded.

5.TABLES

Table 1. Families, species and number of visits of the floral visitors registered in flowering plants in two protected areas at the Atlantic Forest, southeastern Brazil.

Family	Species	Number of interactions
Trochilidae	*Phaethornis eurynome*	547
Trochilidae	*Ramphdon naevius*	137
Trochilidae	*Thalurania glaucopis*	93
Trochilidae	*Clytolaema rubricauda*	2
Heliconiinae	*Heliconius numata robigus*	10
Heliconiinae	*Heliconius ethilla narcaea*	11
Heliconiinae	*Heliconius melpomene nanna*	20
Riodinidae	*Eurybia molochina hyacinthina*	15

Table 2. Traits and network metrics of plant species sampled in two protected areas at the Atlantic Forest, southeastern Brazil. Traits: corolla length, seed set, fitness value and network metrics: degree centrality (*DC*), closeness centrality (CC_w) and interaction selectivity (*d'*).

Plant species	Corolla lenght $\bar{x}$ mm ± SD	Seed set $\bar{x}$ (units) ± SD	Fitness value	*DC*	CC_w	*d'*
Aechmea araneosa L.B.Smith	15.96 ± 0.19	1455 ± 19.76	0.44	1.00	0.13	0.13
Aechmea mutica L.B.Smith	24.24 ± 0.26	279 ± 13.15	0.44	0.75	0.07	0.21
Aechmea pineleana var. Minuta M.B. Foster	10.42 ± 0.04	31 ± 3.04	0.64	0.50	0.04	0.38
Centropogon cornutus (L.) Druce	36.58 ± 1.18	3280 ± 182.74	0.71	0.25	0.10	0.14
Nidularium cariacicaense (W.Weber) Leme	53.82 ± 0.28	1257 ± 58.33	0.37	0.50	0.21	0.09
Nidularium procerum Lindman	45 ± 0.58	160 ± 24.12	0.50	0.50	0.04	0.33
Odontonema dissitiflorum (Nees) Kuntze	40.9 ± 0.33	35 ± 0.48	0.55	0.50	0.03	0.03
Pavonia multiflora A. St-Hil	31.46 ± 0.07	55 ± 2.07	0.43	0.50	0.15	0.09
Quesnelia strobilispica Wawra	50.66 ± 0.23	190 ± 27.66	0.36	0.25	0.01	0.40
Siphocampylus convolvulaceus (Cham.) G. Don	32.92 ± 0.37	680 ± 100.45	0.40	0.25	0.03	0.10
Tillandsia cf. stricta Sol. ex Sims	11.16 ± 0.12	536 ± 35.22	0.60	0.50	0.03	0.07
Vriesea ensiformis (Vell.) Beer	49.52 ± 0.54	150 ± 31.93	0.35	0.25	0.04	0.10
Vriesea scalaris E.Morren	52 ± 0.48	408 ± 84.34	0.38	0.50	0.02	0.14
Vriesea simplex (Vellozo) Beer	64.52 ± 0.30	318 ± 64.36	0.35	0.50	0.01	0.07

Table 3. Results of GLM showing the effect of interaction selectivity and degree centrality metrics on the number of seeds per flower (Model 1) and the effect of interaction selectivity and closeness centrality metrics on the number of seeds per flower (Model 2) in two protected areas at the Atlantic Forest, southeastern Brazil.

Model	Estimate	Std Error	z value	Pr > |z|	AIC_C	BIC	logLik	deviance	R^2
Model 1									
(Intercept)	3.2418	0.3370	9.619	> 0.0001	8983.2	8996.1	487.6	8975.2	0.008
Interaction selectivity (*d'*)	0.0940	0.0157	5.970	> 0.0001					
Degree centrality (*DC*)	0.0801	0.0151	5.300	> 0.0001					
Model 2									
(Intercept)	3.2346	0.3341	9.679	> 0.0001	7879.4	7892.2	-3935.7	7871.4	0.065
Interaction selectivity (*d'*)	-0.081	0.0161	-0.506	0.6130					
Closeness centrality (CC_w)	0.3297	0.0096	34.116	> 0.0001					

6. FIGURES

Figure 1. Location of the Estação Biológica de Santa Teresa and Reserva Biológica Augusto Ruschi, Espírito Santo State, Brazil.

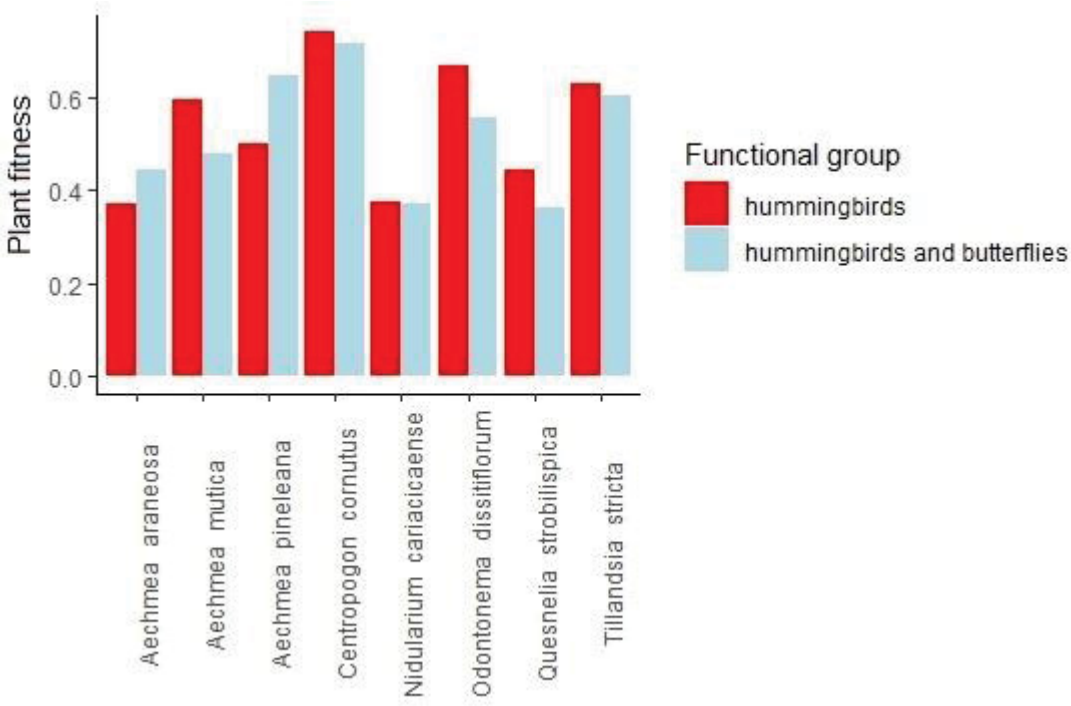

Figure 2. Fitness outcomes of flowers visited by two functional groups: hummingbirds or hummingbirds and butterflies in two protected areas at the Atlantic Forest, southeastern Brazil.

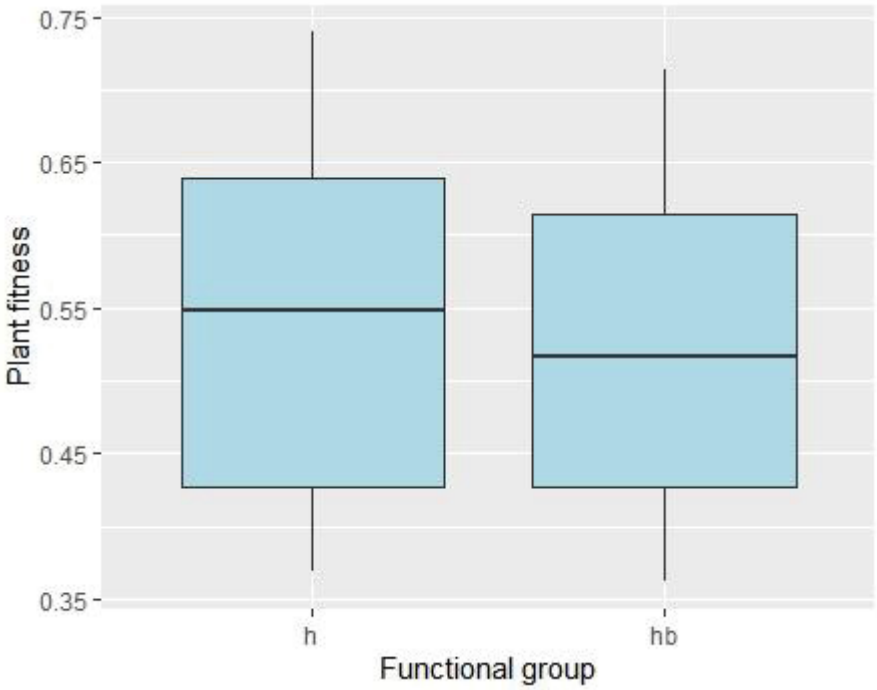

Figure 3. Fitness values for eight plant species visited by hummingbirds or hummingbirds and butterflies in two protected areas at the Atlantic Forest, southeastern Brazil. Within each plant species, the flowers were separated according to the registered floral visitors: "h" represents the group of flowers only visited by hummingbirds and "hb" represents flowers visited by hummingbirds and butterflies. With the number of seeds counted for each group, a fitness value was calculated and compared to each other. The boxplots show the median fitness (black line), the boxes span 50% of the data and the whiskers include the entire range of observed fitness values.

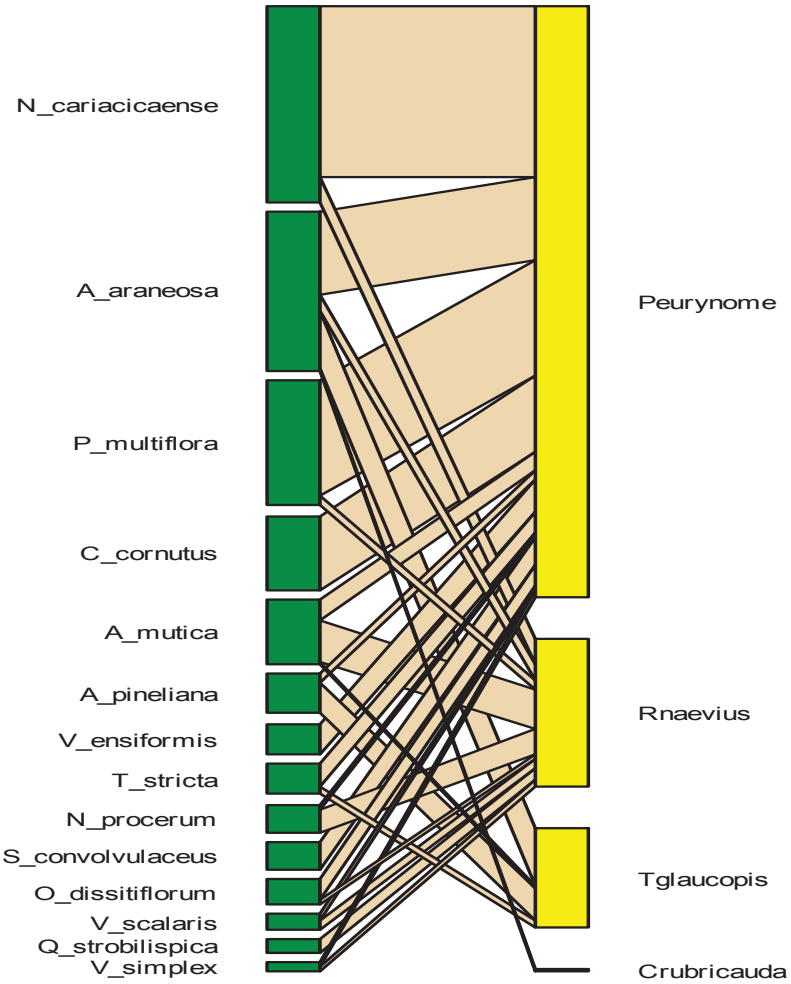

Figure 4. Interaction network between hummingbirds and plant species in two protected areas at the Atlantic Forest, southeastern Brazil. Green boxes represent plant species, yellow boxes hummingbird species. The thickness of the links connecting pairs of boxes indicates the strength of the interaction between species.

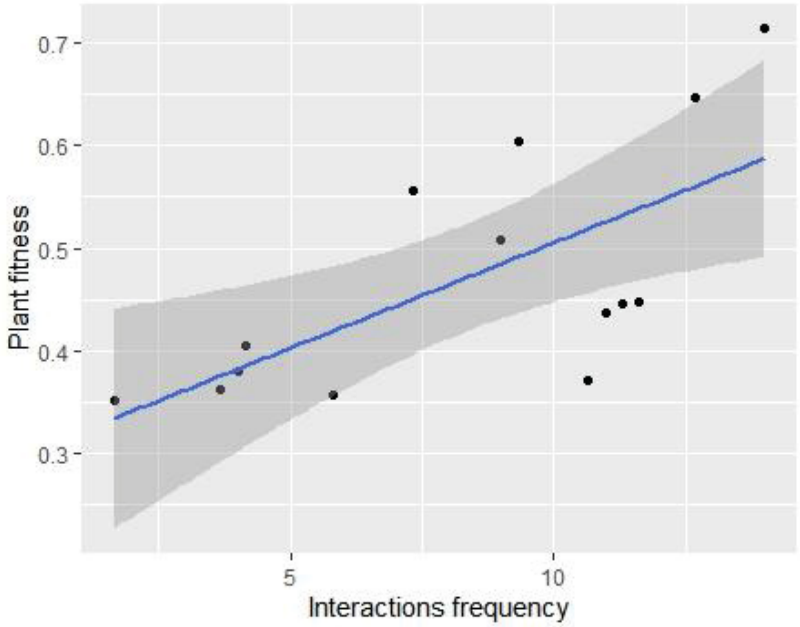

Figure 5. Relationship between frequency of hummingbird visits (x-axis) and plant fitness (y-axis) of 14 species in two protected areas at the Atlantic Forest, southeastern Brazil. The equation resulting from the adjustment of the model is y= 0.02x + 0.30. The slope indicates that plant fitness increases by 0.02 when the plant experiences one more interaction.

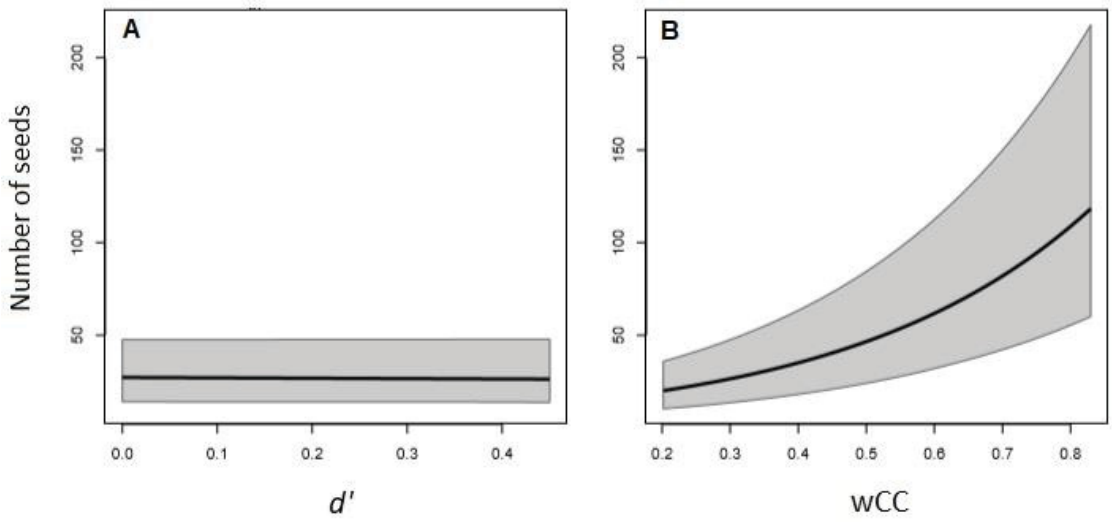

Figure 6. Relationship between interaction selectivity (A) and closeness centrality (B) and number of seeds produced by flowers in the sampled plant species in two protected areas at the Atlantic Forest, southeastern Brazil.

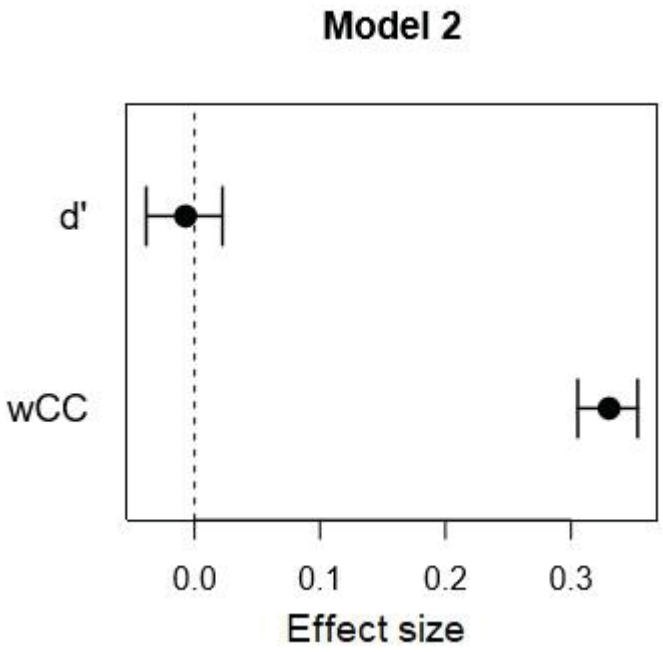

Figure 7. Mean and confidence intervals for the best adjusted model predictors and number of seeds produced by flowers in 14 plant species in two protected areas at the Atlantic Forest, southeastern Brazil. The X axis represents the effect of the predictor on the response variable. The dotted line marks the non-significance of the predictor effect.

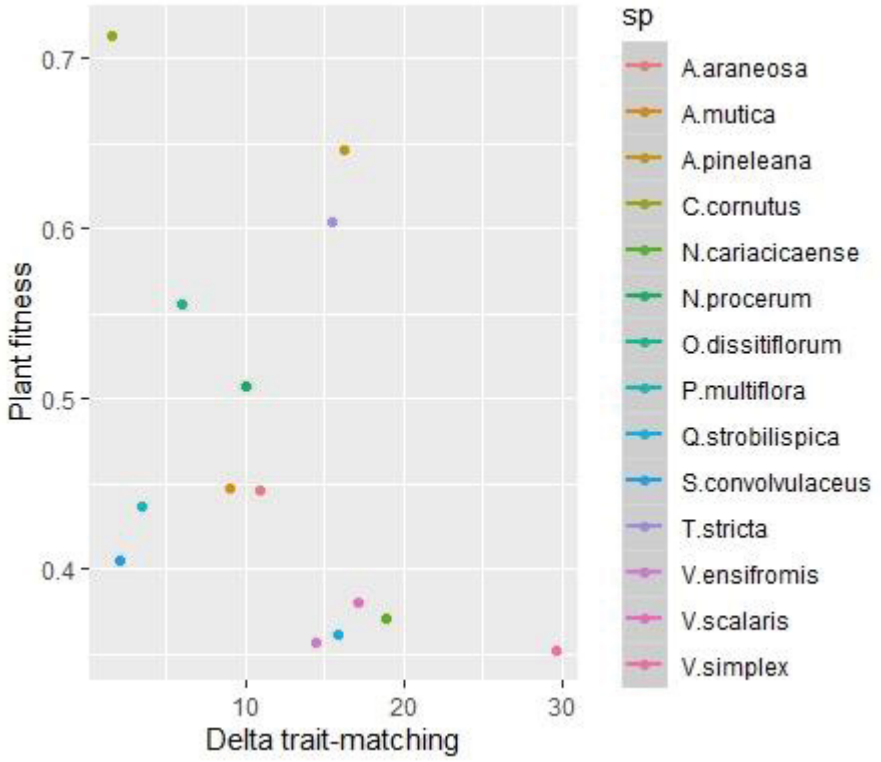

Figure 8. Relationship between differences in plant and hummingbird species trait-matching and plant fitness in a mutualistic network of two protected areas at the Atlantic Forest, southeastern Brazil. Each dot represents one of the 14 observed plant species. The x-axis represents the average in the degree of mismatch between each plant with all its pollinators. Fitness was calculated as a standardized value per plant species, values of trait-matching represent the mean differences between the floral trait (length of the corolla in mm) and pollinators traits (bill length in mm).

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8. SUPPLEMENTARY MATERIAL

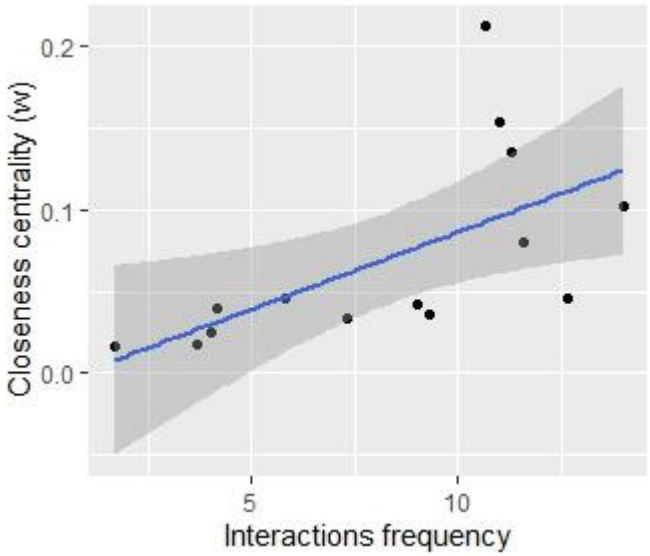

Fig. S1. Relationship between interaction frequency and weighted closeness centrality in 14 plant species in two protected areas at the Atlantic Forest, southeastern Brazil.